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U.S.EPA HPV Chemical Challenge Program

Revised Test Plan for the

Formates Category

Formic Acid* CAS#:64-18-6
Sodium Formate CAS#:141-53-7
Calcium Formate CAS#:544-17-2
Methyl Formate CAS#:107-31-3

Submitted by:
American Chemistry Council
Formic Acid and Formates Panel

Submitted to:
U.S. Environmental Protection Agency

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* Formic Acid is being reviewed as an ICCA chemical and is not formally a HPV chemical.

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Executive Summary

The HPV category "Formates" is proposed and justified to comprise the four HPV chemicals:

Formic acid
Sodium formate
Calcium formate
Methyl formate

These chemicals are used in many diverse applications including agriculture, leather production, grout and concrete mixes, steel making, as solvents and as chemical intermediates.

Experimental evidence is presented to demonstrate that the formate ion is the prime determinate of toxicity for all members of this category. Methyl formate, which is a volatile solvent, may at first glance appear to be an outlier; however, it fits nicely into this category since it is rapidly hydrolyzed *in vitro* and *in vivo* to formic acid and to methanol. Methanol systemic toxicity is known to primarily be a result of formate produced by the biological oxidation of methanol. Pharmacokinetic data indicate that methyl formate is transformed very rapidly into formic acid and methanol in the body with a half-life on the order of several seconds. In the environment, this transformation is facile at neutral pH and increased at slightly basic pH levels.

Although the physicochemical characteristics of these materials vary from volatile liquid to nonvolatile solids, they share most properties that relate to potential impact on the environment and health. All are readily biodegradable and present little or no bioaccumulation or bioconcentration. Although initial environmental distribution varies among these materials, the ultimate fate as carbon dioxide is shared among all members.

Adverse effects on environmental organisms are minimal for the category. Formic acid has specific potential adverse effects by virtue of its strong acidic properties and methyl formate possesses a solvent-narcosis activity prior to hydrolysis. After hydrolysis or

neutralization, all materials converge to the low-hazard formate ion, which itself is readily converted to carbon dioxide in the environment by biodegradation or photo oxidation.

The acute toxicity of all materials is low with no special hazards. As with the environmental effects, formic acid has additional health hazard due to its strong acid properties and methyl formate can produce solvent-narcosis at high concentrations.

Genotoxicity testing results are largely negative but additional information is desired to fulfill the HPV chromosome aberration endpoint of methyl formate and *in vitro* testing is proposed. Low-level exposures to formates are not considered a health concern because formate is a normal component of the human body and is contained naturally in many foods.

After repeated dosing by inhalation or by drinking water, few systemic effects have been observed for formates. A 13-week inhalation study of formic acid in rats and mice provides strong evidence of formate's low systemic hazard. Chronic and multigenerational studies of sodium and calcium formate indicate low chronic, reproductive and developmental hazard; however, these studies are not well documented. Although these studies are of value, the confidence in the results is lower than for the acute hazard. A developmental toxicity study using sodium formate as a model formate is recommended.

Testing Plan

Data for most of the HPV endpoints are either available or can be readily estimated with sufficient certainty for most of the HPV endpoints for these chemicals. Formic acid data needs are being addressed by an EU consortium under the ICCA Initiative. In consideration of animal welfare concerns to minimize the use of animals in the testing of chemicals, the Panel has conducted a thorough literature search for all available data, published and unpublished. It has also performed an analysis of the adequacy of the existing data. Further, it developed a scientifically supportable category of related chemicals and used structure-activity relationship information to fill certain data gaps.

The goal of developing a chemical category is to use interpolation and/or extrapolation to assess chemicals rather than conducting additional testing with specific consideration of animal welfare concerns to minimize the use of animals in the testing of chemicals. The following study is planned to strengthen the data set for toxicological information of formates:

Developmental toxicity for sodium formate

Testing Plan in Tabular Format

Formates Category	Formic Acid*	Calcium Formate	Sodium Formate	Methyl Formate		
HPV Endpoint						
					Codes	
Physical Chemical						
Melting Point	D	D	D	D	*D*	Data Available
Boiling Point	D	NA	NA	D	*E*	Estimate
Vapor Pressure	D	NA	NA	D	*S*	Surrogate
Water Solubility	D	D	D	D	*T*	Testing
Partition Coefficient	D	E	E	D	*NA*	Not applicable
Environmental & Fate						
Photo-Degradation	E	NA	NA	E		
Water Stability	NA	NA	NA	D		
Transport	E	E	E	E		
Biodegradation	D	S	D	D		
Ecotoxicity						
96-Hour Fish	D	D	D	E/D		
48-Hour Invertebrate	D	E	D	E/D		
72-96-Hour Algae	D	E	D	E/D		
Toxicity						
Acute Oral	D	D	D	D		
Acute Inhal	D	NA	D	D		
Acute Dermal	NA	NA	NA	D		
Repeated Dose	D	D	D	S		
Reproductive**	T	S	T	S		
Developmental	T	S	T	S		
Genetic Toxicology *in vitro*	D	D	D	D		
Genetic Toxicology *Clast*	D	S	D	S		

* Formic acid is being addressed under ICCA by an EU consortium.

** Specific reproductive tests will not be conducted as this endpoint may be filled by a combination of a repeated-dose/subchronic study and a developmental toxicity study.

Overview and Justification of Category

Chemicals in the Category

This HPV category is composed of formates and consists of four HPV chemicals.

1. Formic Acid
2. Sodium formate
3. Calcium formate
4. Methyl formate

Formic acid is an ICCA chemical and a separate document is being prepared in the EU for assessment under the OECD/SIDS program. Data on formic acid will be reviewed here because it is the base member of the category and has a rich data set. Other chemicals will be discussed in this document as they relate to the four main compounds. For example, ethyl formate is a close relative of methyl formate and methanol is an environmental and physiological hydrolysis product of methyl formate. Information on biological activity of sodium and calcium ions are also relevant to defining hazard from the two salts.

Rationale for Class

Grouping of similar chemicals into classes is encouraged to conserve resources and reduce animal usage in the HPV Challenge Program. The Formates constitute a category where grouping is readily justified and where viewing the group in this way enhances understanding of the potential toxicity of all members.

There are several possible ways of grouping chemicals in the HPV program to form categories. Ultimately, the best grouping is one that will allow prediction and understanding of the toxicity of similar members of the category based on structure and physical or chemical properties. Similar mechanisms of action and metabolic profiles strengthen the coherence of a category. This grouping of formates fulfills the condition of similar mechanisms and metabolism and provides a logical category. Although the

members have varying physical properties, the contribution of these physical properties to the hazard can be readily estimated from chemical principles.

The primary logic of treating these as a group stems from the high probability that the SIDS toxicity endpoints will be primarily mediated by the formate moiety that all members have in common. Certain differences are recognized which are important to the acute health effects, these include pH, physical state, and likely routes of exposure. On the other hand, the more important long-term toxic effects from low-level exposure are likely related primarily to blood and tissue levels of formate. All four are clearly sources of systemic formate.

Oral exposure to low levels of formic acid, sodium formate or calcium formate are expected to result in essentially identical formate uptakes. The ionization state (neutral or anionic) in the gastro intestinal tract will determine the absorption. The form is determined almost solely by the pKa (3.74) of the formate anion and the pH of the GI tract. Provided excessive amounts of any of these three are not ingested such that the pH is altered (or solubility is delayed), the normal GI pH will result in essentially identical ratios of formic acid:formate. Thus, the absorption, distribution, metabolism, and toxicity of these three formates are anticipated to be identical at low oral exposure levels. Since neither the hydrogen ion, nor the sodium ion, nor the calcium ion is considered highly toxic, differences in toxicity from low-level oral exposure are not anticipated if converted to a mole of formate basis.

Methyl formate is known to be rapidly hydrolyzed by serum and liver esterases (1) and in body fluids (2) to methanol and formate (formic acid). Methanol is rapidly metabolized in the body to formate. Thus, after absorption, hydrolysis and oxidative metabolism of the methanol moiety of methyl formate, all that remains is formate. In practice, the route of exposure for methyl formate is likely to vary from the other materials. For example, since methyl formate is not a strong irritant, is low molecular weight, uncharged and volatile; dermal absorption and inhalation are more likely to be significant routes of exposure.

A PBPK-like toxicokinetic model was recently developed for methyl formate in humans and has been validated using data from volunteers exposed to methyl formate (1). The salient features of this model that validate the inclusion of methyl formate in the formates class are the estimation of the rate constant (K_{MF}) for methyl formate hydrolysis *in vivo*

and the demonstration that the methanol formed is metabolized to formate. The estimated first order rate constant for hydrolysis of methyl formate derived for the model is 6.7 min^{-1}, which corresponds to an *in vivo* half-life of only 6.1 seconds. This indicates that methyl formate hydrolysis is almost instantaneous in the body and it is unlikely that there is significant distribution of methyl formate except as methanol and formic acid. The formed methanol is subsequently oxidized to formate, which is known to be the causative agent for much of the reported methanol systemic toxicity (3). It is therefore prudent when considering systemic toxicity, to treat methyl formate as formate.

In support of a common mechanism of toxicity, the rat oral LD_{50} when calculated in units of milli-equilivants formate per kilogram bodyweight is very similar across the category. Formic acid shows more toxicity, which is anticipated due to its acidity. Methyl formate shows the lowest toxicity on this basis. The lower acute toxicity of methyl formate is possibly due to either/or the slower conversion of methanol to formate altering the formate toxicokinetics or the excretion of methanol or methyl formate via the lungs. In addition, the higher oral LD_{50} of methanol itself (>5000 mg/kg) is in accord with this proposed mechanism for reduced methyl formate oral acute toxicity. Overall, the correlation of LD_{50} with milli-equilivants formate is very good and supports a common mechanism for acute toxicity.

Material	Mol Wt	# Formates	LD_{50}	LD_{50} in meq/kg
Formic acid (4)	46	1	730-1830	16 - 40
Calcium formate (5)	130	2	2700	38
Sodium formate (6)	68	1	>3000	>44
Methyl formate (7)	60	2	1500	50

Another piece of supporting evidence for inclusion of methyl formate in the category comes from the LD_{50} of ethyl formate, which is reported in IUCLID as 1850 mg/kg (8) and as 4490 mg/kg (9). The expectation, based on the assumption that methyl formate is acutely toxic due to its metabolism to formate, is that ethyl formate would have a higher LD_{50} by

about 2-3 fold since it is metabolized to ethanol and formate and the ethanol goes on to acetate rather than formate. The experimental evidence fits reasonably well in this case. Propyl formate, likewise, is reported to have an LD_{50} of 3980 mg/kg in the rat by oral administration also showing a fit of these simple formate esters in the same category with formate salts regarding acute toxicity (10).

A toxicokinetic model for methyl formate exposure and excretion has been recently developed and validated (11). The model indicates that the initial metabolites of methyl formate are formic acid and methanol. Methanol is both excreted and converted to formic acid. Formic acid excretion kinetics in the urine was reported to be controlled by a saturatable urinary reabsorption of formic acid.

It is known that methanol toxicity is largely determined by its metabolism to formic acid (3). The toxic effects of methanol and metabolic acidosis are mainly or completely a result of the extreme elevation of blood formate resulting from metabolism of methanol to formic acid. Formate mediated methanol toxicity also accounts for the species difference of methanol toxicity wherein primates, which are relatively poor at metabolizing formate, are more sensitive to the toxic effect of methanol than are rodents, which metabolize formate more quickly (12). Investigations of methanol toxicity also led to the finding that folate deficiency exacerbates methanol toxicity. The mechanism was determined to be through tetrahydrofolate-mediated metabolism of formate to carbon dioxide. In this mechanism, formate binds to tetrahydrofolate (THF) and the complex is oxidized to carbon dioxide by the enzyme formyl-THF (13). Therefore, lack of sufficient folate leads to a reduced rate of formate metabolic clearance.

Because of the role of formate in methanol toxicity, much of the information derived from the study of methanol is applicable to understanding the toxicity of formates. In addition, this connection of formate with methanol toxicity strengthens the inclusion of methyl formate as a member of the HPV formates category relative to health effects.

Effects on environmental organisms at low levels are expected to primarily be a result of the formate ion. Methyl formate, being an organic ester, is anticipated to have direct acute solvent-like effects on environmental organisms at high concentrations and these narcotic-like effects are considered separately. Environmental hydrolysis of methyl

formate at pH 7 to 9 is known to be a facile process yielding methanol and formic acid (14). At pH 8 and 25 C the hydrolytic half-life of methyl formate in aqueous solution is calculated to be 5.3 hours based on the published K_b. Under typical environmental conditions, formic acid that is formed by hydrolysis will react with water to quantitatively produce formate anion. Methanol that is produced will be biologically oxidized via formaldehyde to formate and finally carbon dioxide. Thus, methyl formate fits in the category relative to environmental effects.

Available data for aquatic toxicity support the proposed categorization and are given in the table below. As discussed above, methyl formate is anticipated to differ from the others due to the ester moiety dominating the acute toxic effects. Formic acid is anticipated to differ by virtue of its acidity. Calcium ion is known to be of low aquatic toxicity (15); therefore, calcium formate should fit into the paradigm adequately.

Material	LC_{50} or EC_{50} (mg/L)		
	Fish	Daphnids	Algae
Formic acid (4)	46-175	120-150	25
Calcium formate (5)	>1000	ND	ND
Sodium formate (6)	>5000	>1000	~1000
Methyl formate (7)	120	>500	190-240

In summary, the category approach is well supported for the proposed "Formates" category comprised of formic acid, sodium formate, calcium formate and methyl formate. The environmental and health effects of the ionic formates are primarily determined by the formate moiety. Methyl formate is rapidly converted to formic acid and methanol, which is subsequently converted to formate. Much of the environmental and health effects data developed for any member of the category will apply across the category. The category approach is justified to save resources including the use of experimental animals.

Production, Uses and Exposures

All of these formates are produced or imported over a million pounds per annum into the United States. The uses and potential exposures vary across the category. It should be noted that formates are naturally occurring in the body and in many foods. The introduction to the NTP 13-Week study report summarizes the natural occurrence of formic acid as follows: "Formic acid, first described by Fisher in 1670 in the products resulting from the distillation of red ants (16), occurs in both natural and man-made sources in the environment. A constituent of ant, wasp, and bee venom, formic acid also occurs in mammalian muscle tissue, sweat, and urine. It is found in plants, such as in the needles of the Douglas fir, and in unripened grapes, peaches, raspberries, strawberries, petitgrain lemon, and in bitter orange (17). It also is present in many foods (18), e.g., fruits (20 - 40 ppm), fruit juices (30 - 100 ppm), fruit syrups (650 - 1630 ppm), honey (20 - 2000 ppm), wines (1 - 340 ppm), coffee, roasted (1350 - 2200 ppm), coffee, extracts (2000 - 7700 ppm), evaporated milk (30 - 400 ppm), and cheese (20 - 200 ppm) (19)." (20).

Methyl formate has also been detected in foods. Reports describe its occurrence in tomatoes (21), apples (22), and coffee (23). Therefore, oral exposure through ingestion of common foodstuffs will contribute to human exposures.

Formic Acid

In the *Kirk-Othmer Encyclopedia of Chemical Technology* (24) it is stated that there are three main processes used to produce formic acid. The first is by acid-hydrolysis of formate salts that are in turn by-products of other processes. The second is as a co-product with acetic acid in the liquid-phase oxidation of hydrocarbons and the third is by carbonylation of methanol to methyl formate, followed by direct hydrolysis of the ester or through formamide. Worldwide production of formic acid was estimated at 330,000 tones per annum in 1988 with US production estimated at 13,000 tones per annum (25).

Formic acid is used in textile dying and finishing, as a chemical intermediate, in leather processing, in rubber manufacture, as a catalyst in hydrocarbon-formaldehyde resins &

phenolic resins and as a plasticizer for vinyl resins. It also is reportedly used in the electroplating industry, as an antiseptic in wine and beer brewing, as a preservative in animal feed additives, as a component of cleaning solutions, as a wire stripping compound, in the preparation of bare wires for soldering, as a laundry sour and as an oil well acidifying agent (26).

The primary use worldwide is as a silage additive. This use is more prevalent in Europe than the US. Formic acid application to fresh-cut grasses prior to ensilation enhances the nutritional value of the produced silage. Lactic acid production is enhanced while the undesirable butyric acid production is avoided. Formic acid can also be used as an additive in animal feeds where it has anti-bacterial activity. Use as a chemical intermediate includes the preparation of formate esters used in flavors and fragrances and in the synthesis of aspartame. U.S. Production in 1975 was reported as 28 million kg (24).

The United States usage pattern of formic acid was described in 1965 by SRI International as 55 percent used in textile dyeing and finishing; 15 percent as an intermediate for formates; 10 percent in leather tanning; and 20 percent in miscellaneous applications (26). A more recent estimate of usage from the Chemical Products Synopsis in 1985 indicates shifting usage with textile dyeing and finishing at 21 percent, 20 percent in pharmaceuticals, 16 percent in rubber intermediate, 15 percent in leather and tanning treatment, 12 percent in catalysts, and 18 percent in miscellaneous uses including oil well acidizing (26). The 2001 Chemical Economics Handbook does not break the usage pattern into percentages but suggests a similar distribution of uses. It also adds the following uses, in the manufacture of epoxidized soybean oil and as an active ingredient in commercial cleaning products (27).

Exposure to formic acid may occur by inhalation, dermal absorption or ingestion. As formic acid is strongly irritating, it is assumed that exposure by the inhalation or the dermal route is self-limiting. Oral ingestion of foodstuffs with naturally occurring formic acid content is not thought to be a health concern and ingestion is an unlikely route relative to industrial exposure where most of the US production is consumed. No information on exposure levels was available for review.

In 1976, under contract to the FDA, the Federation of American Societies for Experimental Biology (FASEB) produced a "GRAS Document" covering formic acid,

sodium formate and ethyl formate. The conclusion of this report was that the use of formic acid and sodium formate as an ingredient of paper and paperboard food packaging material does not present a hazard (28).

The FDA allows the use of formic acid as a food additive permitted for direct addition to food for human consumption as a synthetic flavoring substance and adjuvant in accordance with the following conditions: 1) the quantity added to food does not exceed the amount reasonably required to accomplish its intended physical, nutritive, or other technical effect in food, and 2) when intended for use in or on food it is of appropriate food grade and is prepared and handled as a food ingredient (29). In addition, the FDA permits the use of formic acid as a preservative in hay crop silage in an amount not to exceed 2.25% of the silage on a dry weight basis or 0.45% when direct-cut. The top foot of silage stored should not contain formic acid and silage should not be fed to livestock within 4 weeks of treatment (30).

Sodium Formate

Sodium formate is produced by the reaction of carbon monoxide with sodium hydroxide and as byproduct in the production of pentaerythritol. U.S. Production in 1975 was reported as 15 million kg. (31).

Sodium formate is used as an intermediate in the production of formic acid, oxalic acid and a few other chemicals. It is used in electroplating and textile production, in the tanning of leather and as a reducing agent (31). Other uses are for gas scrubbing, as an oil-well drilling fluid additive and a small quantity is used as an ingredient in liquid detergents. The major current uses are in leather tanning, gas scrubbing and as an oil-well drilling fluid additive (32).

Sodium formate is affirmed as GRAS by the FDA as a constituent of paper and paperboard used for food packaging (33).

Exposure to sodium formate is restricted to workers in chemical plants producing the material, chemical workers using it as an intermediate, textile workers, electroplaters, leather tanners, workers using it for gas scrubbing and as an oil-well drilling fluid additive, and minor consumer dermal exposure from its use in liquid detergents. Since it is not volatile, inhalation exposure is restricted to conditions where particulate material

may be suspended in air and inhaled. As an ionic solid, dermal exposure will not result in significant systemic exposure.

Calcium Formate

Calcium formate is prepared from the high-temperature and high-pressure reaction of calcium hydroxide and carbon monoxide (34). It is also available industrially as a by-product from the preparation of pentaerythritol and other polyhedric alcohols and of disodium dithionite (24).

Calcium formate is used as a preservative for silage and as a food preservative. It also finds use as a component of drilling fluids and lubricants, as a binder for fine-ore briquets, in the tanning of leather and in flue gas scrubbing (34). Calcium formate is added to feed and premixes for piglets and calves as an organic acid to stabilize the digestive process (35). It also finds use as a nonchloride accelerator used to reduce the setting time of concrete and similar materials (36). Use in grouts and concrete products are major current uses in the U.S. for calcium formate.

Exposure to calcium formate appears to be restricted to workers in chemical plants using it as an intermediate, or as a component of grout and concrete mixes, farm workers using it to treat silage and potentially workers preparing and dispensing feed where it may be used as an additive. Since it is not volatile, inhalation exposure is restricted to conditions where particulate material may be suspended in air and inhaled. As an ionic solid, dermal exposure will not result in significant systemic exposure.

Methyl Formate

Reported methods for the preparation of methyl formate are the reaction of methanol, carbon monoxide and steam, over a charcoal or sodium methoxide catalyst at 200 degrees C and 200 atmospheres of pressure; by esterification of formic acid and methanol and by heating methyl alcohol with sodium formate and hydrochloric acid (37).

Methyl formate is used as a solvent and a chemical intermediate, a fumigant and larvicide for tobacco, dried fruits, cereals and other foods, and as a high-boiling refrigerant (37). Other reported uses are as a solvent for cellulose acetate (38) and as a catalyst and

binding agent for core sand in the production of mold cores in iron foundries where it has replaced dimethylethylamine (39).

Human Experience and Considerations

General

Humans are known to accumulate toxic formate more easily than non-primate experimental animals due to their reduced capacity to metabolize formate to carbon dioxide. Much of this information comes from the study of methanol toxicity where humans have greater sensitivity than most animals and show ocular toxicity while rodents do not. The enhanced methanol sensitivity in large part is a result of the biological oxidative conversion of methanol to formic acid resulting in a metabolic acidosis (and ocular effects) not seen in most lower-animals. The accumulation of formate in humans is due to a relative deficiency in formate metabolism as compared to most experimental animals, related partly to a low hepatic tetrahydrofolate (H4 folate) levels in humans. There is an excellent correlation between hepatic H4 folate and formate oxidation rates within and across species. Humans possess low hepatic H4 folate levels and demonstrate low rates of formate oxidation and the accumulation of formate after methanol exposure (40).

Formic Acid

In the industrial setting formic acid is know to be a severe skin, mucus membrane, eye and respiratory tract irritant; however, few other adverse effects have been definitively associated with industrial exposures (26). Since formic acid is naturally occurring in many foods and as formate is a normal constituent of intermediary metabolism (41), low level systemic exposure is not likely to result in adverse effects.

Intentional ingestion (overdoses) are reported to produce salivation, vomiting (which may be bloody), a burning sensation in the mouth and pharynx, diarrhea, and severe pain. Circulatory collapse may follow, causing death (42). Ellerhorn's Medical Toxicology notes that formic acid ingestions are unique in their ability to cause death after a

prolonged course of classical acid-induced gastrointestinal damage. Ingestions of less than 10 grams by children have led to superficial oropharyngeal burns with the children recovering. In adults, ingestions exceeding about 50 grams were generally fatal with lesser doses resulting in superficial oropharyngeal burns, hematemesis, hepatotoxicity, ulcerations and perforation of the gastrointestinal tract (43).

Twelve farmers exposed to formic acid for eight hours in silage making were examined for effects on calcium excretion and renal ammoniagenesis. Eight of the subjects were exposed below 9 mg/m^3 (MAK value) and four were exposed at or above this level. Exposure was associated with increased renal ammoniagenesis and urinary calcium excretion at 30 hours post exposure. It was speculated that these biochemical effects could be explained by the interaction of formic acid with the oxidative metabolism of renal tubular cells. The authors concluded that current hygienic exposure limits might not entirely protect formic acid exposed individuals from renal effects (44).

Exposure limits for formic acid are (45):

- ACGIH TLV: 5 ppm; 9.4 mg/m3 (as TWA); 10 ppm; 19 mg/m3 (as STEL) (ACGIH 1996)
- MAK: 5 ppm; 9 mg/m3; (1995).
- OSHA PEL: TWA 5 ppm (9 mg/m3)
- NIOSH REL: TWA 5 ppm (9 mg/m3)
- NIOSH IDLH: 30 ppm

Sodium Formate

Reports of human experience to exposures of sodium formate are very limited. In Gosselin et al. (46) the entry for formic acid and salts, lists for sodium formate: "sodium formate appears to have a low toxicity (10 g by mouth without ill effects in man)". This report is consistent with the relatively low toxicity of formic acid especially considering sodium formate is not acidic. As noted under formic acid human experience, formate is a normal constituent of intermediary metabolism and low-level systemic sodium formate exposure is probably inconsequential.

No occupation exposure standards were located for sodium formate.

Calcium Formate

No human experience information was available for inclusion and no occupation exposure standards were located. As noted in other sections, formate is a normal constituent of intermediary metabolism and low-level systemic calcium formate exposure is probably inconsequential.

Methyl Formate

Exposure to methyl formate is believed to occur primarily by inhalation due to its volatility. Dermal exposure is also expected to result in systemic uptake; however, dermal exposure is thought to be very limited as methyl formate, to the best of our knowledge, is only used in industrial settings.

Methyl formate vapor exposure has been reported to produce nasal and conjunctival irritation, retching and CNS depression (37). Recently exposure of human volunteers at 100 ppm was shown to be associated with an increase in the subjective feeling of fatigue without impairment of neurobehavioral performance (47). A study of exposure and neurobehavioral endpoints was recently reported in a foundry. Although the measured exposure exceeded the MAC value of 400 ppm on some occasions, there were no measurable neurobehavioral changes in this group of 10 workers (48).

Exposure limits for methyl formate are (49):

- ACGIH TLV: 100 ppm; 246 mg/m^3 STEL 150 ppm (ACGIH 1996).
- OSHA PEL: TWA 100 ppm (250 mg/m^3)
- NIOSH REL: TWA 100 ppm (250 mg/m^3) ST 150 ppm (375 mg/m^3)
- NIOSH IDLH: 4500 ppm

Physicochemical Information

The physicochemical properties of this class are dependent on the physical form and ionization status. Properties required under HPV are either known or can be easily extrapolated for all class members.

Methyl formate is the only true neutral organic molecule in this class, it is a low-boiling volatile liquid, and its physicochemical properties relevant to HPV are known.

Formic acid is also a liquid but much less volatile. On dissolution in water it partially ionizes according to its pKa and the resulting pH of the solution to formate ion. The IUCLID summary notes that there is a discrepancy in the octanol-water partition coefficient values in the literature. This is not surprising as the partitioning is pH and hence concentration dependent. The conclusion, however, is that the equilibrium favors water for both the ionized and non-ionized forms; thus, the Po/w is not definitively known (and may not be directly measurable) but the available information is considered adequate for the HPV Program endpoint.

Both calcium and sodium formate are water-soluble salts of formic acid. As salts, they are solids and essentially non-volatile. Because they are salts of formic acid, the octanol-water partition coefficients are pH dependent and not definitive without specification of concentration or pH. They both favor water and are sufficiently well established for the needs of HPV.

	Physical State	Boiling/Melting Point	Vapor Pressure (20)	Water Solubility	Log Ko/w
Formic acid (4)	Liquid	100.6 C	42 hPa	Miscible	-0.54
Methyl formate (7)	Liquid	32.3 C	644 hPa	300 g/L	-0.21
Sodium formate (6)	Solid	253 C	Nil	550 g/L	ND
Calcium formate (5)	Solid	>300 C	Nil	160 g/L	-2.47

Summary and Recommendations for Physicochemical Information. All parameters have adequate data for the purposes of HPV. No additional testing is recommended.

Fate Information

Distribution in the environment is anticipated to be the same for formic acid and its sodium and calcium salts provided the pH is equivalent in the environment. The proper calculation of distribution at neutral pH values is to use the properties of the ionized form. The Mackay level III model contained in EPIWIN suggests that the majority of any of the formates entering a waste water plant will be contained in the effluent. In the case of methyl formate, about 10 percent of the material will likely be lost to air. As all of these formates are readily biodegradable, the actual effluent output will be dependent upon several factors with residence time, temperature and acclimation predominating. Relative to the HPV program, it can be concluded that formic acid, sodium formate and calcium formate will distribute almost exclusively to water where biodegradation will occur with no bioaccumulation. Methyl formate will distribute to water and air where it will photodegrade (air) or biodegrade (water, it may also hydrolyze prior to biodegradation) with little or no bioaccumulation.

As a category, the ultimate fate of all of these materials in the environment is dependent primarily upon the fate of the formate ion. Methyl formate is known to break down rapidly to formic acid and methanol. Under aerobic conditions, methanol will oxidize through formate to carbon dioxide. Sodium and calcium cations are stable in the environment but are considered innocuous after dilution. Formate anion is known to be readily biodegradable (50).

All four materials have been tested for aerobic biodegradation and found to be "readily biodegradable" by the OECD criteria (see robust summaries). Although the quality of the tests varies, the consistency and structural similarity indicate that the formate moiety and the methyl moiety are readily biodegraded.

Photodegradation in the atmosphere is reduced by the category approach to a consideration of the photodegradation of formic acid and methyl formate. Sodium formate and calcium formate are not volatile; however, under acidic conditions they will be converted to volatile formic acid that will undergo atmospheric photodegradation.

The rate of reaction of atmospheric hydroxyl radical with formic acid is known and at the default (5.0×10^5 molecule per cubic centimeter) concentration of atmospheric hydroxyl

radicals, a $t_{1/2}$ of 35 days is predicted (based on a 24-hour day, see robust summaries for formic acid).

Methyl formate has an experimentally measured reaction rate constant with hydroxyl radicals (given in the APOWIN 1.90 tables comparing estimated to actual values) and using the current EPA default of 1.5 E^6 hydroxyl radicals per cubic centimeter a $t_{1/2}$ of 47 days (based on a 12-hour day)(7).

Abiotic degradation in water is an important mechanism for loss of Methyl formate based on its estimated hydrolytic half-life in water at 20°C of 67 hours at pH 7 and 40 minutes at pH 9. The attached robust summary contains specific information on the determination of the hydrolysis rate constant and the appropriateness of this methodology to estimation of the environmental hydrolysis rate.

Summary and Recommendations for Fate

All fate parameters for all members of the category have adequate information to fulfill the HPV Program requirements. No testing is recommended.

Effects on the Environment

Fish, Invertebrates and Aquatic Plants

The effects of formates on fish, invertebrates and aquatic plants either are known or can be predicted by the category approach and the structures of the materials. In addition a major environmental impact study of sodium formate was conduced by Transport Canada at the Halifax airport in support of the use of sodium formate as a deicing compound on runways (51). The results of this study indicated that the use of sodium formate over the winter of 1991-2 at Halifax International airport had minimal effects on the adjacent vegetated soils or on monitored test streams. In this study, multiple tons of materials

were used on a taxiway and extensive environmental quality monitoring was conducted on surface (including aquatic organisms) and groundwater.

Available data for aquatic toxicity are given in the table below. As discussed above, methyl formate is anticipated to differ from the others due to the ester moiety dominating the initial acute toxic effects prior to hydrolysis. Formic acid is anticipated to differ by virtue of its acidity. It can be calculated from the K_a that, without buffering, a 10 mg/L solution of formic acid will have an approximate pH of 3.7 and the expected pH is 3.2 at 100 mg/L. It is apparent from the sodium formate data that the formate ion itself has a low order of toxicity and since calcium ion is known to be of low aquatic toxicity (52) it is evident that calcium formate will also have low order of toxicity toward these aquatic species.

Material	LC_{50} or EC_{50} (mg/L)		
	Fish	Daphnids	Algae
Formic acid (4)	46-175*	120-150	25
Calcium formate (5)	>1000	NA	NA
Sodium formate (6)	>5000	>1000	~1000
Methyl formate (7)	120	>500	190-240
(Methanol) (53)	>1000	>1000	>1000

* Without pH adjustment, with pH adjustment the LC_{50} is the same as sodium formate.

NA means not available

Concern with the aquatic data include both the hydrolysis and volatility of methyl formate. Environmental hydrolysis of methyl formate at pH 7 to 9 is known to be a facile process yielding methanol and formic acid (54). At pH 8 and 20 C the hydrolytic half-life of methyl formate in aqueous solution is calculated to be 6.7 hours based on the published k_b. Under typical environmental conditions, formic acid produced by hydrolysis will react with water to give formate ion that will biodegrade to carbon dioxide. The methanol produced by hydrolysis will be biologically oxidized via formaldehyde to formate and finally carbon dioxide. It can be argued that the static toxicity test, where hydrolysis products are allowed to form, is a more realistic test of the

acute toxicity of this material in the environment; however, in situations where there is a continuous influx of material in to the environment, a flow through test might be more appropriate relative to a localized area of a waterway. Logically, with the low Ko/w of methyl formate and the high rate of abiotic and biotic degradation, accumulative effects are not anticipated to be important for fish and daphnids and a combination of QSAR modeling with available static results is considered acceptable for the aquatic toxicity endpoints. A more in depth discussion of the estimation of Methyl formate aquatic toxicity and the issues surrounding this estimation is provided in Appendix 1 of this document.

In addition, the EPA ECOSAR model for esters gives a predicted 96-hour LC/EC_{50} of 132 mg/L for fish, 4500 mg/L for daphnids and 9 mg/L for algae. These are in accord with the observed static results. The experimental algae result of an IC_{50} in the range of 190-240 mg/L is easily reconciled. This is the expected apparent result over a 96-hour study if there is an initial strong inhibition of growth followed by rapid hydrolysis of methyl formate to the essentially non-inhibitory methanol and formate. The EPA ECOSAR model does not take hydrolysis into consideration; therefore, the predicted value may be in accord with the experimental value for algal inhibition. Based on the available test results and the known environmental fate of formic acid and methanol, the aquatic hazard of methyl formate is sufficiently characterized for the purposes of the HPV Program.

In summary, the formates as a category have low aquatic hazard with the exception of pH effects of formic acid and an initial moderate toxicity of methyl formate to fish and algae which is followed by a rapid hydrolysis to the less toxic products of methanol and formate.

Summary and Recommendations for Environmental Effects

All environmental effect parameters for all members of the category have adequate information to meet the HPV Program requirements. No testing is recommended.

Health Effects

Acute Oral Toxicity

As with the environmental effects, the acute oral toxicity of formate itself appears to be very low; however, the acidity associated with formic acid appears to increase the acute oral toxicity of this substance and the solvent-narcotic effect of methyl formate appears to increase its acute toxicity.

Formic Acid

Several acute oral toxicity tests of formic acid have been conducted giving LD_{50} values between 730 mg/kg and 1830 mg/kg (55). The lowest LD_{50} value (730 mg/kg) is selected as the key study as it is both the lowest and the study followed the OECD 401 guideline using four dose levels and groups of 5 rats of each sex (56).

Sodium and Calcium Formate

The acute oral LD_{50} for both these materials is high and similar showing the low level of acute toxicity associated with the formate ion. Calcium formate has three studies conducted giving LD_{50} values of 2650, 2560 and 3050 mg/kg (5). According to the IUCLID 2000 document, sodium formate had an OECD 401 guideline study conducted in 1989. The result of this unpublished study is an LD_{50} >3000 mg/kg (57). Neither additional details nor the study report were available for review.

Methyl Formate

The key study for methyl formate acute oral toxicity, giving an LD_{50} of 1500 mg/kg, was conducted in 1979 using five Sprague-Dawley rats of each sex at doses of 464, 681, 1000, 1470, or 2150 mg/kg. All high-dose animals died, 2/5 males and 2/5 females died in the 1470-mg/kg dose groups. Surviving rats gained weight and did not appear to have

delayed effects. All deaths occurred within one hour of dosing. The time course of death, clinical observations and post-mortem findings are consistent with solvent-narcotic activity resulting from the bolus dose of methyl formate overwhelming the hydrolytic capability of the test animals, being the cause of death (7).

Summary and Recommendations for Acute Oral Toxicity

For the purposes of the HPV Challenge Program, the acute oral toxicity is sufficiently characterized for all members of the category. No further testing is recommended.

Acute Inhalation Toxicity

Acute inhalation data are available for three of the four materials in this category.

Material	LC_{50}
Formic acid	7.4 mg/L
Calcium formate	No data
Sodium formate	>0.64 mg/L
Methyl formate	>21 mg/L

Formic Acid

The acute inhalation LC_{50} for formic acid is reported as 7.4 mg/L in IUCLID-2000 with the notation that it was a BASF test using 10 animals of each sex per group with a 14-day observation period. No details or report were available for review (58).

Sodium Formate

The acute inhalation toxicity of sodium formate was determined to be > 0.67 mg/L in a 1990 GLP study using the solid aerosol. The study was conducted at what was considered the maximum attainable inhalation concentration. Milled test material was used and a MMAD of 5.4 microns was measured in the chamber. All animals survived the 4-hour exposure and the 14-day observation period. The only adverse effects notes were lacrimation and nasal discharge (59).

Calcium Formate

No inhalation studies were located, based on the sodium formate results and the minimal toxicity of calcium salts, calcium formate can be considered to have low inhalation hazard.

Methyl Formate

The 4-hour inhalation LC_{50} of methyl formate was determined to be > 21 mg/L in a GLP study conducted using measured concentrations of test material (60). The study was conducted using a single concentration level and adverse effects were minimal. This study is described in detail in the robust summaries. Additional supporting studies are also available and are cited in the robust summary (7).

Summary and Recommendations for Acute Inhalation Toxicity

For the purposes of the HPV Challenge Program, the acute inhalation toxicity is sufficiently characterized for all members of the category. No further testing is recommended.

Acute Dermal Toxicity

The acute dermal toxicity of methyl formate in rats was found to be >4000 mg/kg in a 1979 unpublished study (61). Clinical signs including staggering and irregular breathing indicated dermal absorption and sublethal effects at this dose level. This is supported by a 1990 screening-level dermal toxicity study of methyl formate sponsored by Hoechst Celanese in which 0/4 treated rabbits died at a dermal dose of 5,000 mg/kg (62).

No testing is indicated for the other materials since formic acid is corrosive to the skin and the other two materials are salts of materials having low toxicity.

Summary and Recommendations

Acute dermal toxicity information relevant to the HPV Program is known, can be estimated with sufficient confidence, or is irrelevant for all members of the category. No testing is recommended.

Repeated Dose Toxicity

Formic Acid

The National Toxicology Program has conducted 2-week and 13-week inhalation studies with formic acid. The results show little systemic toxicity and the primary adverse effects involve the nasal epithelium. The 13-week NOAEL for rats and mice was reported to be 32 ppm. It was concluded in the abstract of the report: "Overall, the effects of formic acid were consistent with those of irritant chemicals administered by inhalation exposure. The no-observed-adverse-effect level (NOAEL) for respiratory injury was 32 ppm in rats and mice. There was no significant evidence of systemic toxicity in these studies." (20).

A feeding study using pigs was conducted with duration of approximately 90-days examining the effect of feeding Ca/Na-formate (50:50 weight basis) or K-diformate (30% potassium, 35.4 % formic acid and 34.6% formates) at 0, 0.6 or 1.2% of the diet to growing-finishing pigs (63). The K-diformate has previously been shown to be an effective growth promoter in diets of both weaning pigs (64) and growing-finishing pigs (65). There was no effect of the Ca/Na-formate on growth or any other measured parameter. K-diformate, added on top of the basal diet, significantly increased the growth rate of the pigs. There were no adverse effects on the health status of pigs fed K-diformate and an examination of the stomachs at necropsy revealed no effect on stomach keratinization or ulceration. Additional studies revealed that feeding 1.2% K-diformate to pigs decreases the coliform bacteria level in the gastrointestinal tract. The presumed mechanism of action was reportedly partially explained by reduction of the population of

gut coliform bacteria leading to reduced metabolic needs of gut bacteria and improved availability of dietary nutrients for the animal.

Sodium Formate

A one-and-a-half-year drinking-water study has been conducted using sodium formate. The results are only available as a brief keynote address and describe a study using six rats per group exposed to one percent sodium formate in the drinking water for one and a half years. The conclusion was that no toxicity was detected (66). The pig-feeding studies listed under formic acid also contained sodium formate and suggest a lack of toxic effect at moderate dose levels after repeated oral exposure.

Calcium Formate

A lifetime drinking water study has been conducted with calcium formate in the drinking water at 0, 0.2, or 0.4% (150-200 mg/kg/day in the lowest dose according to the authors). Six rats per group were used and the results are only summarized in keynotes or presented briefly in a table in the case of body weight gain. Macroscopic and histological examinations were conducted upon the natural death of the animals. No significant clinical or pathologic changes (growth or organ functions) were detected in any dose group; in particular, there were no disorders of the ocular fundus. The study includes several generations (up to 5). At the beginning, 8 males and 24 females were used (66). A summary of this study may also be found in the IUCLID document for formic acid. The pig-feeding studies listed under formic acid also contained calcium formate and suggest a lack of calcium formate induced toxic effect at moderate dose levels.

Methyl Formate

No data were found for methyl formate; however, information is available for its degradation products methanol and formic acid. Dahl et al. (67) have demonstrated that carboxylesterases are very active in the respiratory tract of rats, rabbits and hamsters. These authors concluded, “The foregoing calculations, based upon the experimental results, indicate that inhaled esters may be largely converted to hydrolysis products in the

nasal cavity". Beyond the nasal cavity of rats, these authors found that the rat lung had about half the carboxylesterases activity (on a per mg. protein basis) as the nasal epithelium and the liver had about twice the carboxylesterases activity of nasal epithelium. Thus, ample additional esterase activity is available to rapidly hyrdolyse esters that make it past the nasal epithelium. Recently, Niehlen and Droz developed and validated a toxicokinetic model of methyl formate absorption, metabolism and excretion in humans. The first-order rate constant for hydrolysis of methyl formate was estimated by fitting the toxicokinetic model to individual experimental data from 36 methyl formate exposed individuals. The range of values obtained from these subjects was from 4.3 to 7.3 min^{-1}. The value selected for use in the model is 6.7 min^{-1}, which corresponds to a half-life of 6.1 seconds (11). These studies indicate that the systemic hazard of methyl formate inhalation can be established from the results of methanol and formic acid systemic exposure studies.

In the NTP formic acid studies, discussed under formic acid, there was no evidence of systemic toxicity after inhalation exposure of rats or mice to formic acid vapor up to 500 ppm for 2 weeks (5 days a week, 6 hours a day) or up to 128 ppm for 13 weeks (5 days a week, 6 hours a day).

There are several studies demonstrating the low toxicity of methanol to experimental animals. Exposure of rats to methanol vapor up to 5000 ppm for 4 weeks (5 days a week, 6 hours a day) resulted in only mucoid nasal discharge while monkeys tolerated 5000 ppm under these conditions with only a slight increase in the spleen weight of females (68). Rats exposed to methanol vapor daily for 20 hours a day for up to two years or mice exposed for up to 18 months at 10, 100 or 1000 ppm showed minimal treatment related effects (69). Monkeys exposed to methanol vapor for 21 hours a day in a series of range-finding subacute studies showed no adverse effects at 3000 ppm or below but demonstrated adverse clinical signs at 5000 ppm and above. After daily chronic exposure of up to 7 months for 21 hours a day, 1000 ppm was found to be a LOAEL. In a 30-month inhalation study, monkeys exposed daily for 22 hours a day demonstrated slight liver and kidney effects at 1000 ppm, kidney effects at 100 ppm and CNS effects which were considered transient at 10, 100 and 1000 ppm. (69). Gavage administration of methanol to rats for 90 days at 0, 100, 500 or 2500 mg/kg/day produced few adverse effects. Some organ weights were affected at the high dose without corresponding histopathological changes. The NOEL was 500 mg/kg (70).

In addition to these studies of formic acid and methanol, a recent 4-week study on the close analog methyl acetate has been conducted. In this study, male and female rats were exposed by inhalation to vapors of methyl acetate for 6 hours a day, 5 days a week for 4 weeks at concentrations of 0, 75, 350 or 2000 ppm. The only adverse effect found after this exposure was degeneration of the nasal epithelium in 19/20 treated high-dose rats. The 350-ppm level was considered a NOAEL. Blood levels of methyl acetate were determined immediately upon cessation of the study and no methyl acetate could be found in the blood of exposed animals. This indicates the experimental animals effectively and rapidly hydrolyzed inhaled methyl acetate (71).

Summary and Recommendations for Repeated Dose Toxicity

Sufficient data are available within this category to meet the requirements of the HPV Challenge Program. Methyl formate has not been directly studied but studies of the other formates, methanol and methyl acetate provide evidence that significant adverse systemic effects from repeated administration of methyl formate are unlikely. The only adverse effect anticipated from methyl formate inhalation exposure at high levels is degeneration of the nasal epithelium. No further testing is recommended.

Genetic Toxicity *In Vitro*

All four materials have been found negative in the Ames test as shown in the table.

Results of Ames Testing on Formates

Material	Result	Guideline Study	Year	GLP	Ref
Formic acid[#]	-/-*		1975, 1983		20, 72, 73
Calcium formate	-/-	Yes	1983	Yes	74
Sodium formate	-/-	No	1975	No	75
Methyl formate	-/-	No	1989	Yes	76

* -/- indicates negative in the presence of absence of liver S9 fraction

Formic acid has multiple negative Ames tests

Other *In Vitro* Assays

Formic acid

A published cytogenetic assay using CHO-K1 cells produced ambiguous results for chromosome aberrations. The unbuffered or unneutralized acid was clastogenic at pH values around 6.0 (10-14 mM) and cytotoxic at and below pH 5.7 (12-16 mM). Clastogenicity is stopped by neutralization with sodium hydroxide or by increasing the buffer concentrations in the incubation medium. The authors conclude from this that it is not the substance as such that induces chromosome damage but that the damage is due to the acid pH of the incubation medium as a nonspecific effect. The study was conducted basically in accord with the OECD 473 guideline "*In Vitro* Mammalian Chromosome Aberration Test" (77).

Two sister chromatid exchange assays have been conducted using formic acid. Both produced negative results. One utilized Chinese hamster V79 cells at formic acid concentrations of 0.4, 0.6, 1.0 and 2.0 mM with and without an activation system (78). The second utilized human lymphocytes at a formic acid concentration from 29 - 460 ug/ml (0.63 - 10 mM) with an activation system (79).

An *E. coli* reverse mutation assay without activation produced slightly positive results. In this 1951 report, the number of bacteria was varied while the test substance concentration remained at almost the same level. The survival rate was reduced with a decrease in the bacterial count (from 100% at 1.5×10^9 bacteria up to 2.8% at 2.6×10^7). In parallel, the number of mutations was reduced with an increase in the survival rate (80). Other bacterial (Ames) tests produced negative results as indicated above.

Formic acid was reported to be negative in a SOS chromotest. The test was conducted with and without an activation system and used 3-5 concentrations at up to 100 mM (81).

Sodium Formate

A bacteria reverse-mutation assay of sodium formate produced negative results (75). This is consistent with the other formates that were negative in the Ames test.

Sodium formate was tested for clastogenicity by Morita et al. (77) who tested formic acid neutralized with sodium hydroxide or sodium bicarbonate (both of which produce sodium formate) in cultured CHO-K1 cells. This peer-reviewed report describes an OECD 473 like study in which it was demonstrated that sodium formate is not clastogenic to these cells. The study was further strengthened by the demonstration that neither acetic acid nor lactic acid was clastogenic after neutralization to sodium acetate and sodium lactate although they displayed clastogenic activity at acid pH.

Sodium formate was reported positive in a mouse lymphoma assay in both the presence and absence of metabolic activation. The study is reported with no details in the Chemical Carcinogenesis Research Information System file (maintained by the National Library of Medicine) (82); no report has been located describing the conditions of this study. Based on the year of the study (1982 or earlier) and the lack of positive mutagenicity data for other tests and members of this category, this positive result is considered suspicious, as colony sizing was probably not conducted. The current OECD 476 (adopted 21 July 1997) guideline requires colony sizing to confirm the positive result. Likewise, the 1994 Mammalian Cell Gene Mutation Assays Working Group report (83) states that "Ability to recover small colonies must be convincingly demonstrated when using the L5178Y TK mouse lymphoma assay". In addition, the 1995 report by Coombs et al (84) also emphasizes the importance of colony sizing to the acceptability of mouse lymphoma results. Thus, this report is considered unreliable.

Genetic Toxicity *In Vivo*

Formic Acid

A Drosophila SLRL test was performed using oral (feed) or inhalation exposure. The mutation result was positive after inhalation exposure and administration via the diet with

mutation rates of 1.31 and 1.11% as compared with the control limit of 0.15% in each case. If the pH was buffered to 7.5 in the feeding study, there was no increased mutation rate. The positive effect was likely due to the pH of the acid form used in the testing (85, 86).

Sodium Formate

Formic acid neutralized with glycine-NaOH buffer was tested in a Drosophila SLRL test using oral exposure. After feeding for the entire larval stage of development, males did not show an increase in mutation rate at 0.1% formic acid neutralized. Feeding the acid form without neutralization produced a statistically significant positive result (86).

Methyl Formate

No studies of methyl formate itself are available; however, as it is known that methyl formate is rapidly hydrolyzed to formic acid and methanol, the data for these compounds is relevant. Formic acid data is provided in this document and the methanol data can be found in the HPV document for methanol (EPA RTK website). Methanol has been extensively studied for potential genotoxicity and the weight of evidence indicates lack of genotoxicity. In addition, the close analog methyl acetate was not genotoxic in a rat micronucleus assay at inhalation doses of up to 2000 ppm (71).

Summary and Recommendations for Genetic Toxicology

The genetic toxicology data set for formates suggest a lack of genotoxic potential. Positive results that have been obtained were attributed to low pH values in the test systems as has been reported in the literature for other acids (87). No further testing is recommended.

Reproductive Toxicity

Reproductive toxicity studies of formates are limited; however, a multigeneration drinking-water study with calcium formate has been conducted.

Formic Acid

There are data from the 13-week NTP inhalation study covering some reproductive systems that do not indicate any obvious reproductive toxicity.

In the rat study it is stated that for the SMVCE parameters "There were no effects of exposure to formic acid on measures of sperm motility, density, or testicular or epididymal weights, and no changes were seen in the length of the estrous cycle."

In the 13-week mouse study, it is concluded "There were no adverse effects of formic acid exposure on reproductive parameters evaluated in male or female mice (Appendix C). Sperm motility was somewhat lower in the exposed groups compared to controls, but the values for controls were rather high, and the values for exposed mice fall well within the historical range for control mice."

In rats, no histopathological, gross or organ weight changes were noted in male or female reproductive organ systems after 2-weeks of exposure at up to 500 ppm or after 13-week exposure at up to 128 ppm.

In mice, no histopathological, gross or organ weight changes were noted in male or female reproductive organ systems after 2-weeks of exposure at up to 500 ppm or after 13-week exposure at up to 128 ppm.

Calcium Formate

Results of a three-generation drinking water study at 0 or 200 mg/kg/day calcium formate in the drinking water have been published (66). Number, weight and length of offspring did not differ in treated animals from controls. An additional study of identical design at 400 mg/kg/day was stated in the report as producing no adverse effects. In these studies, a portion of the offspring was also sacrificed shortly after birth for evaluation of developmental toxicity. No statistical differences in organ or bone abnormalities were

found. The growth of treated offspring was similar to controls. Presentation of data is limited to the 200 mg/kg dose group.

Sodium Formate

No studies were identified except it was stated in the calcium formate multigenerational study publication that a similar study was underway with 1% sodium formate (ca 1000 mg/kg/day). This study was stated to be ongoing for one and a half years and no effects indicating that this treatment was harmful had been observed. Data, however, were not presented and results showing that this study was completed were not found in the open literature (66).

Methyl Formate

No studies of methyl formate were found; however, data on formic acid and methanol are relevant in estimating the reproductive hazard of methyl formate as these are the primary *in vivo* metabolites. The formic acid data are discussed above and methanol has been well studied for reproductive toxicity.

There are conflicting reports on the effects of methanol on testicular function. Cameron et al. (88, 89) reported that male rats exposed repeatedly by inhalation (260, 2600 or 13000 mg/m^3) to methanol demonstrated reduced serum levels of testosterone. Cooper et al (90) also reported lowered testosterone levels, decreased testis weight, and decreased numbers of morphologically normal sperm after gavage dosing for 21 days with 1,600 mg/kg/day methanol but not with 800 mg/kg/day.

In contrast to these reports, Lee et al. (91) reported that rats exposed by inhalation to 260 mg/m^3 for up to 6 weeks did not show any changes in serum testosterone, or in testis or seminal vesicle weights, or several *in vitro* biochemical parameters. In addition, normal or folate deficient rats exposed to 1,040 mg/m^3 methanol for 20 hours a day, 7 days a week for up to 13 weeks did not show any effects on testicular morphology, testis weights or seminal vesicle weight. Older folate deficient rats, however, exposed to methanol did have an increased incidence of age-related testicular degeneration.

Summary and Recommendations for Reproductive Toxicity

Limited data are available for formates regarding reproductive toxicity. Low-level exposures are not of concern because formate is a normal component of human metabolism. Higher-level exposure is unlikely to result in significant reproductive toxicity based on the data from formic acid and calcium formate. Methyl formate reproductive toxicity may be defined by any representative formate and the extensive methanol data showing minimal risk from low to moderate methanol exposure. For EPA HPV Program purposes, the present data from subchronic studies with examination of reproductive organs will be considered sufficient for the formate category provided a definitive developmental toxicity is conducted on a representative formate.

Developmental Toxicity

The only *in vivo* developmental toxicity study known for formates is the calcium formate multigenerational study described above in the reproductive toxicity section. In the publication of this study, it was also reported that sodium formate injected into eggs at 5, 10 or 20 mg, had no effect qualitatively or quantitatively on the malformation spectrum of frequency of the resultant chicks (66). Both the rat study and the chicken study showed no evidence of developmental toxicity but are not considered adequate in design or reporting. No definitive studies on developmental toxicity of any of these formates were located.

Studies of methanol developmental toxicity are relevant to the formate category since it is a metabolite of methyl formate and is itself metabolized to formate. Developmental toxicity studies of methanol suggest the potential to cause developmental toxicity in experimental animals. An inhalation study in mice at 1,000, 2,000, 5,000, 7,500, 10,000, or 15,000 ppm methanol for 7 hr/day on days 6-15 of gestation was conducted in which the NOAEL for developmental toxicity was 1000 ppm (92). In this study, methanol doses of 5,000 ppm or higher caused significant increases in the incidence of exencephaly and cleft palate. Doses of 2,000 ppm or higher induced increases in the frequency of cervical rib formation or ossification sites lateral to the seventh cervical vertebra. Maternal toxicity was only reported at 7500 ppm and above. This investigation was extended to

examine the effect of methanol exposure at various times during development. It was concluded that the conceptus is most sensitive to the effects of inhaled methanol during gastrulation and early organogenesis (93). Other developmental toxicity investigations have been conducted and provide results that are broadly similar. For example, the inhalation NOAEL for developmental effects in the rat is 5000 ppm (94). The developmental toxicity of methanol was also shown to be exacerbated in mice provided a folic-acid deficient diet (95, 96). This observation indicates that formate, which is oxidized by a tetrahydrofolate pathway, is at least partly responsible for the adverse developmental effects of methanol. It is not known if formate alone can produce the same effects in a standard developmental toxicity study.

The developmental toxicity of formate and formic acid have been investigated using whole embryo culture of rats and mice (97). It was reported that both formic acid and sodium formate are approximately equally embryotoxic and are four to eight times more potent than methanol at the same molar concentration. In a study designed to elucidate further details on the relationship of formate to methanol developmental toxicity, the combined effect of methanol and formic acid on rat embryos in whole embryo culture was examined (98). The authors concluded that the combined exposure had less effect on the cultured embryos than was predicted by simple additively and therefore the mechanism of activity is likely different for the two agents. This result also demonstrates that the dysmorphogenic effects observed in the presence of methanol are not likely due to a synergistic combined effect of methanol with its metabolite formate.

Gavage administration of sodium formate (750 mg/kg) to pregnant CD-1 mice on gestational day eight did not result in exencephaly in spite of the fact that it produced a peak blood formate level similar to that produced following a 6-hour exposure to 15,000 ppm methanol (99). This observation led the authors to conclude that developmental toxicity of methanol by inhalation in CD-a mice was not a result of the accumulation of formate but a direct result of the methanol concentration.

Developmental toxicity studies of Methyl formate have not been conducted; however, as it is metabolized *in vivo* to formic acid and methanol, the data on methanol and formate developmental toxicity serve to characterize the developmental toxicity of methyl formate.

Overall, the studies conducted using formate to investigate methanol-induced developmental toxicity have been inconclusive and additional information would help provide further understanding of the potential of high doses of formate to cause developmental toxicity.

Summary and Recommendations for Developmental Toxicity

Limited data are available for this category regarding developmental toxicity. Low-level exposures are not of concern because formate is a normal component of human metabolism. Higher-level exposure may be selectively hazardous to the conceptus. A definitive study on a representative formate – such as sodium formate – where the formate dosage may be maximized without causing narcosis or acidosis is recommended as a means of completing HPV Program requirements.

Overall Summary

Relative to the HPV program, most of the requested screening data for this category of chemicals are available or can be reliably estimated. Some of the existing studies are not of modern quality but data from other members of the class increases the overall confidence of the data sets for fate, environmental effects and acute health effects. The consistency of the available data confirms the validity of grouping these materials into a category.

Although multi-generational studies have been conducted for formate salts without producing developmental effects, the studies are not sufficiently robust to fill the developmental data endpoint. The fact that formates are a normal constituent of human metabolism further reduces the concern of low-level hazard; however, testing of a representative formate for developmental toxicity would add to the confidence of the hazard evaluation for this category.

Recommended testing for the category is developmental toxicity testing in rats of a representative formate. Sodium formate is recommended as the preferred salt to be tested for developmental toxicity as pH effects and irritation will be minimized, and as sodium is the most common extracellular cation. It is further recommended that a sodium control group be added to account for any role of excess sodium cation on development. In addition to these recommendations, it should be kept in mind that a European consortium is addressing the data-set for formic acid under the ICCA program.

References

1 Nihlen A, Droz PO. Toxicokinetic modelling of methyl formate exposure and implications for biological monitoring. Arch. Occup. Environ. Health 73:479-487 (2000)

2 Browning E, Toxicity and Metabolism of Industrial Solvents, New York: American Elsever, 1965 p 588 (peer reviewed) – as cited in Hazardous Substance Data Base, update 0f 02/08/2000

3 McMartin, KE et al. Methanol Poisoning I. The role of formic acid in the development of metabolic acidosis in the monkey and the reversal by 4-methylpyrazole. Biochem. Med. 13:319 (1975)

4 See Robust Summaries for Formic Acid

5 See Robust Summaries for Calcium Formate

6 See Robust Summaries for Sodium Formate

7 See Robust Summaries for Methyl Formate

8 Jenner PM et al. Food Cosmet. Tox. 2: 327-243 (1964)

9 Symth et al. Arch. Ind. Hyg. 10:61-68 (1954)

10 RTECS (FCTXAV 2,327,1964)

11 Nihlen, A. and Droz, NA. Toxicokinetic modelling of methyl formate exposure and implications for biological monitoring. Arch Occup. Environ. Health 73(7):479-487 (2000)

12 McMartin, KE et al. Methanol Poisoning V. Role of formate metabolism in the monkey. J. Pharmacol. Exp. Ther. 201:564-72 (1977)

13 Marcus, WL. Methanol: Drinking water health advisory, Office of Drinking Water, USEPA J Env. Pathol. Toxicol. Oncol. 12:115-138 (1993)

14 See description of Kb and pH dependent kinetics in the robust summary for methyl formate fish and daphnia toxicity.

15 IUCLID-2000 document for Calcium Formate

16 Windholz, M ed. The Merck Index, tenth edition, 1983 p 605

17 NCI Summary Data Sheet on Formic Acid, prepared for the National Cancer Institute for SRI International under contract No. NO1-CP-95607, 1981

18 Gley, P, and Courtois, JE Report from the commission on nutrition concerning the uses of formic acid and formates as agents for preservation of food products. Bull. Acad. Natl. Med. 151, 598-601 (1967)

19 Tracor Jitco, Inc. Scientific literature reviews on generally recognized as safe (GRAS) food ingredients - formic acid and derivatives. Prepared by Tracor Jitco, Inc., for FDA. NTIS Publication PB-228 558. Washington: U.S. Department of Commerce, National Technical Information Service. 1974

20 National Toxicology Program, NTP Toxicity Report Series 19, Formic Acid administered by inhalation to F344/N Rats and B6C3F1 Mice, July 1992

21 Sohn TW et al; Han'guk Nonghwa Hakhoechi 31: 292-7 (1988); as cited in Hazardous Substance Data Base, record for methyl formate revision of 8/8/2001

22 Rhoades JW; J. Agri. Food Chem. 8: 136-41 (1960); as cited in Hazardous Substance Data Base, record for methyl formate revision of 8/8/2001

23 Neubeller J, Buchloh G; Mitt Klosterneuburg 36: 34-46 (1986); as cited in Hazardous Substance Data Base, record for methyl formate revision of 8/8/2001

24 Kirk-Othmer Encyclopedia of Chemical Technology, Fourth Edition, vol. 11 John Wiley & Sons, publisher, 1998

25 Ullmann's Encyclopedia of Industrial Chemistry, Sixth Edition, 2001 Electronic Release.

26 Hazardous Substance Data Base, National Library of Medicine, Formic acid (record 1646), revision of 8/9/2001

27 SRI International, Chemical Economics Handbook, 2001

28 Evaluation of the Health Aspects of Formic Acid, Sodium Formate, and Ethyl Formate as Food Ingredients. Federation of American Societies for Experimental Biology, Bethesda Md; prepared for Food and Drug Administration, Bureau of Foods, NTIS Doc PB-266 282, 1976

29 21 CFR 172.515

30 21 CFR 573.480

31 Hazardous Substance Data Base, National Library of Medicine, Sodium formic (record 744), revision of 8/9/2001

32 Communication from Hercules Inc, October 2001

33 21CFR186.1756

34 Hazardous Substance Data Base, National Library of Medicine, Calcium formate (record 5019), revision of 8/8/2001

35 Degussa-Huls Internet information
http://www.methionine.com/default.cfm?activity=products&event=calciumformate

36 See http://www.fhwa.dot.gov/infrastructure/materialsgrp/acclerat.htm also
http://www.fabcrete.com/admixtures.html

37 Hazardous Substance Data Base, National Library of Medicine, Methyl formate (record 223), revision of 8/8/2001

38 Hawley's Condensed Chemical Dictionary, Sax and Lewis eds. Van Nostrand Reinhold, New York NY, p770 (1987)

39 Nihlein, A and P.O. Droz. Toxicokinetic modelling of methyl formate exposure and implications for biological monitoring. Int Arch Occup Environ Health. 2000 Sep;73(7):479-87

40 Tephly TR; Life Sci. 48 (11): 1031-41 (1991)

41 Clayton, G. D. and F. E. Clayton (eds.). Patty's Industrial Hygiene and Toxicology: Volume 2A, 2B, 2C: Toxicology. 3rd ed. New York: John Wiley Sons, 1981-1982. 4908 (as cited in HSDB)

42 American Conference of Governmental Industrial Hygienists, Inc. Documentation of the Threshold Limit Values and Biological Exposure Indices. 6th ed. Volumes I,II, III. Cincinnati, OH: ACGIH, 1991. As cited in Hazardous Substance Data Base.

43 Ellenhorn, MJ et al., *Ellenhorn's Medical Toxicology*, second edition, Williams and Wilkins: 1997, p 1087

44 Liesivuori J, Laitinen J, Savolainen H Archives of Toxicology, 66: 522-524 (1992)

45 International Chemical Safety Card 0845, http://www.cdc.gov/niosh/ipcsneng/neng0485.html, accessed October 3, 2001

46 Gosselin, R.E., H.C. Hodge, R.P. Smith, and M.N. Gleason. Clinical Toxicology of Commercial Products. 4th ed. Baltimore: Williams and Wilkins, 1976, p. II-71

47 Sethre, T. et al., Experimental Exposure to Methyl Formate and its Neurobehavioral Effects. Int Arch. Occup. Envir. Hlth. 73:401-9 (2000)

48 Sethre, T. et al. Isopropanol and methylformate exposure in a foundry: exposure data and neurobehavioral measurements. Int. Arch. Occup. Environ. Health 73:528-36 (2000)

49 International Chemical Safety Card 0664, http://www.cdc.gov/niosh/ipcsneng/neng0664.html, accessed October 3, 2001

50 Multiple studies: see IUCLID Formic Acid Document

51 Transport Canada, Environmental Impact from the use of Sodium Formate at Halifax International Airport. Volume I: Final Report. Prepared by Nolan Davis & Associates November 1992

52 IUCLID 2000 document for Calcium Formate

53 IUCLID 2000 document for Methanol

54 See description of Kb and pH dependent kinetics in the robust summary for methyl formate fish and daphnia toxicity.

55 IUCLID document prepared by BASF, revision of May 2000, see robust summaries for updated version of this document

56 Huels AG, unpublished study # 0359 dated 02/05/1985.

57 IUCLID 2000 Document, Sodium Formate, European Chemical Bureau, created 18-Feb-2000.

58 IUCLID 2000 Document, Formic Acid, European Chemical Bureau, created 18-Feb-2000.

59 BioDynamics Inc. An Acute Inhalation Toxicity Study of C-1261 in the Rat. Project # 89-8232, Sponsored by Hoechst Celanese Corp, May 31 1990

60 BioDynamics Inc, Project 87-8030, An Acute Inhalation Toxicity Study of C-1160 in the Rat. Sponsored by Hoechst-Celanese, 1/06/1988

61 BASF AG, Abteilung Toxikologie, unveroeffentlichte Untersuchung (78/495), 22.05.1979

62 BioDynamics Inc, Acute Dermal Toxicity, Rabbits C-1160, sponsored by Hoechst Celanese, 2/28/1990

63 Overland, M et al. Effect of dietary formates on growth performance, carcass traits, sensory quality, intestinal microflora, and stomach alterations in growing-finishing pigs. J Anim Sci. 78:1875-84 (2000)

64 Paulicks, BR et al Dose effects of potassium diformate on the performance of growing piglets. Agribiol. Res. 49:318-326 (1996)

65 Kirchgesser M et al Effects of supplementation of diformate complexes on growth and carcass performance of piglets and fattening pigs in response to application time. Agribiol. Res. 50:1-6 (1997)

66 Malorny, G.: Z. Ernaehrungswiss. 9, 332-339 (1969)

67 Dahl,A.R., Miller,S.C., and Petridou-Fischer, J. Carboxylesterases in the respiratory tract of rabbits, rats and Syrian hamsters. Toxicol. Lett. 36:129-136 (1987)

68 Andrews,L.S., J.J.Clary, J.B.Terrill,and F.Bolte. Subchronic inhalation toxicity of methanol. J Toxicol. Environ. Health 20:117-124 (1987)

69 New Energy Development Organization (NEDO). Toxicological research of methanol as a fuel for power station. Tokyo, Japan. September,1986

70 USEPA 1986, see IRIS record for summary of study

71 Unpublished study, Celanese Ltd. 1999

72 Haworth, S. et al. Environ. Mutagen. 5, Suppl. 1, 3-142 (1983)

73 Kleindienst, T.E. et al. Environ. Sci. Technol. 19, 620-627 (1985)

74 Bayer AG data, Report No. 17969, 4/25/1989

75 Huels Report No. 88/210 (unpublished) 1975

76 Microbiological Associates Inc, Salmonella/Mammalian Preincubation Mutagenicity Assay with a Closed Phase Induction System. Report T8837.502002. 09/27/1989. Sponsored by Hoechst Celanese

77 Morita, T. et al. Mutation Research 240, 195-202 (1990)

78 Basler A. et al. Arch. Toxicol. 58, 10-13 (1985)

79 Sipi, P. et al. Mutation Research 279, 75-82 (1992)

80 Demerec, M. et al. Am. Nat. 85, 119-136 (1951)

81 von der Hude, W. et al. Mutation Research 203, 81-94 (1988)

82 Chemical Carcinogenesis Research Information System. Record 1037 – year, 1982 (maintained by the National Library of Medicine)

83 Mutation Research 312:235-9 (1994)

84 Coombs et al. The use of L5178Y mouse lymphoma cells to assess the mutagenic, clastogenic and aneugenic properties of chemicals. Mutagenesis 10:403-8 (1995)

85 Stumm-Tegethoff, B.F.A. Naturwissenschaften 24, 646-647 (1964)

86 Stumm-Tegethoff, B.F.A. Theor. Appl. Genetics 39, 330-334 (1969)

87 Kirkland DJ, Muller L, Interpretation of the biological relevance of genotoxicity test results: the importance of thresholds. Mutation Research 464:137-47 (2000)

88 Cameron, AM et al Circulating concentrations of testosterone, leuteinizing hormone, and follicle stimulating hormone in the rat after inhalation of methanol. Arch. Toxicol. Supp 7:441-43 (1984)

89 Cameron, AM et al Circulating steroids in male rats following inhalation of methanol. Arch. Toxicol. Supp. 8:422-24 (1985)

90 Copper, RL et al. Effect of methanol on hormonal control of testes in male rats. The Toxicologist 10:211 (1990)

91 Lee, E et al. Effects of methanol vapors on testosterone production and testis morphology in rats. Toxicology and Indust. Health 7:261-75 (1991)

92 Rogers JM et al. The Developmental Toxicity of Inhaled Methanol in the CD-1 Mouse, with Quantitative Dose-Response Modeling for Estimation of Benchmark Doses. Teratology 47:175-188 (1993)

93 Rogers JM, Mole ML Critical periods of sensitivity to the developmental toxicity of inhaled methanol in the CD-1 mouse. Teratology 55:364-372 (1997)

94 Nelson B.K., Brithwell W.S., Mackenzie D.R., Khan A., Burg J.R., Weigel W.W., Goad P.T. Teratological assessment of methanol and ethanol at high inhalation levels in rats. Fundam Appl. Toxicol. 5: 727-736 (1985)

95 Sakanashi TM et al. Influence of folic acid intake on the developmental toxicity of methanol in the CD-1 mouse. Teratology 49:368 (1994)

96 Sakanashi TM et al. influence of maternal folate status on the developmental toxicity of methanol in the CD-1 mouse. Teratology 54:198-206 (1996)

97 Andrews JE et al. Developmental toxicity of formate and formic acid in whole embryo culture: a comparative study with mouse and rat embryos. Teratology 51:243-51 (1995)

98 Andrews JE, et al. Effects of combinations of methanol and formic acid on rat embryos in culture. Teratology 58:54-61 (1998)

99 Dorman DC et al. Role of formate in methanol-induced exencephaly in CD-1 mice. Teratology 52:30-40 (1995)

Appendix I. Hydrolysis and Aquatic Toxicity of Methyl Formate

This appendix has been prepared for the Formates U.S.EPA HPV Formates Category document and presents a more through discussion of some of the issues surrounding the estimation of Methyl formate aquatic toxicity.

As methyl formate hydrolyzes fairly rapidly in water (half-life 6.7 hours at pH 8), aquatic toxicity studies are difficult to perform and their results are difficult to interpret as the actual exposure is to a mixture of methyl formate, methanol and formic acid. Even with a flow-through system there will be a significant concentration of methanol and formic acid that will vary as a function of the flow rate. Ideally, estimation of the toxicity using a validated SAR and comparison of this value with the aquatic toxicity of the hydrolysis products, methanol and formic acid, is the preferred way to assess aquatic hazard of pure methyl formate. From a practical viewpoint, the aquatic toxicity of Methyl formate is better assessed by using data from the hydrolysis products formic acid and methanol.

Veith et al.1 studied the toxicity of 29 industrial esters to fish and concluded that the toxicity of these compounds to fish is greater than predicted using the Ko/w with the narcosis QSAR model. They developed a QSAR model for esters based the data from these 29 esters. Although they did not specifically include any formate esters in the study, no reason for excluding these simple esters was given or is obvious from chemical or toxicological principles. The U.S. EPA has adopted this validated SAR relationship for esters noting that it restricted to acetates, benzoates, dicarboxylic aliphatics and phthalates derived from aliphatic alcohols (ECOSAR Documentation). No rational for exclusion of formate esters is given nor is apparent inspecting the wide variety of esters that were used to develop the QSAR. It appears instead, that the limitation stems from the fact that no formate esters were tested. To help bridge this gap, the ECOSAR predicted toxicity of Ethyl formate was compared to its experimentally determined toxicity and the

1 Veith, G, D. DeFoe and M. Knuth. Structure-Activity Relationships for Screening Organic Chemicals for Potential Ecotoxicity Effects. Drug Metabolism Reviews 15:1295-1303 (1985).

relationship was found adequate.2 Therefore application of the QSAR to formates is considered an appropriate means to estimate the toxicity of methyl formate.

When applied to methyl formate, the ECOSAR-derived fish 96 hour LC_{50} is predicted to be 132 mg/L and the daphnid EC_{50} predicted to be 4500 mg/L. When toxicity values are estimated using the neutral organics model predicted values for fish and daphnids are 7322 and 6753, respectively. The esters model accounts for the "excess toxicity" displayed by esters over what would be expected from a neutral organic acting by simple narcosis. Although no rationale is known for this excess toxicity of esters, one could speculate that it is a result of the enhanced uptake of esters (based on $K_{o/w}$) as compared to the acid and alcohol components alone. It is expected that esterases in the organisms would cause rapid hydrolysis of the esters resulting in 1) a higher internal concentration of acid and alcohol than would be absorbed if exposure was to the acid and alcohol forms, 2) and absorption-excretion equilibrium disruption due to a lower internal concentration of ester that would "pull" more ester into the organism, 3) a metabolic acidosis as the hydrolysis product is acidic. This hypothesis is supported by the low reactivity of esters with non-specific biological macromolecules.

As there are some experimental data available for methyl formate it is useful to compare these results with the predictions from modeling and to consider the effect of hydrolysis on pH. In the study that is summarized for methyl formate on fish, the level of 215 mg/L (3.58 mM) resulted in 100% mortality within 24 hours. A level of 100 mg/L (1.66 mM) resulted in 30% mortality observed at 48 hours and later. Lower concentrations produced no mortality. Unfortunately, pH values after exposure were not recorded for the methyl formate exposures; however, these can be estimated from experimental data available for formic acid. Both studies were conduced in the same laboratory with water of the same hardness and "acid capacity". As the hydrolysis of methyl formate produces formic acid and methanol, the formic acid will dissociate in water resulting in acid conditions. In the studies with formic acid, a 1.0 mM (46 mg/L) solution of formic acid had an initial pH of

2 The ESOSAR predicted 96-hour LD50 for fish is 88.8 mg/L while the experimental value is 230 mg/L (as determined by Veith and cited in the ECB IUCLID 2000 document for Ethyl formate). The base-catalyzed hydrolysis constant (k_b) for Ethyl formate was determined by Hammett (Humphreys, H and Hammett, L. . J. Am Chem Soc. 78: 521-524, 1956) to be 1.98 L/mol-sec at 19.76° C; therefore, hydrolysis in 96 hours is not an issue.

4.3, but resulted in no mortality. A 2.18 mM (100 mg/L) solution gave a pH of 3.3 and was associated with 100% mortality as soon as 1 hour after initiation of exposure. In the case of neutralized formic acid, no mortality was observed at 100 mg/L.

It could be speculated that the fish mortality produced by methyl formate (3.58 mM) resulted from hydrolysis of the methyl formate to formic acid and a resulting severe pH reduction. It is, however, highly unlikely that this occurred, as the kinetics of hydrolysis are pH dependent. As the pH drops, the rate of hydrolysis falls ten-fold with every unit of pH. Based on a $t_{1/2}$ of 67 at pH 7, once the pH drops to 6.0 the $t_{1/2}$ becomes 670 hours for the remaining methyl formate hydrolysis. It is apparent the pH could not fall below 5.0 in the 96-hour incubation as a result of methyl formate hydrolysis. We also know from the formic acid experiments (on the same species of fish), that the fish tolerate a pH of 4.3 without mortality. Therefore, the fish mortality at 215 mg/L methyl formate was not a result of pH reduction resulting from hydrolysis. Furthermore, as the hydrolysis of as little as 1 mM of the methyl formate would produce a pH of 4.3 (based on experimental data from formic acid), it can be concluded that less than one-third of the methyl formate at the highest concentration was lost to hydrolysis. At lower levels (such as the 100 mg/L, 1.66 mM level) the proportion potentially subject to hydrolysis is somewhat greater but still this estimation of methyl formate concentration is within the typical variations routinely observed in inter-laboratory and inter-species variation in the estimation of the LC_{50} to freshwater fish.

Although it can be concluded that hydrolysis was not a major source of error in the available data for methyl formate aquatic toxicity, the issue of volatility still remains. The low value of the Log Ko/w (-0.17) and high water solubility confers a moderately low Henry's Law Constant (experimental value) of 2.23 X 10-4 atm-m3/mole. This suggests moderate volatility from water and EPIWIN predicts a t1/2 for volatilization from a model lake at 96 hours. Based on this prediction, the concentration of methyl formate in the fish and daphnia tests probably remained in a range where the test was reasonably robust during the 48-hour daphnid test and the 96-hour fish test. In addition, as the material is not anticipated to bioconcentrate to any degree, the initial 24 to 48 hours of exposure is expected to provide a good approximation of the 96-hour mortality.

From a practical standpoint, between the rapid hydrolysis and biodegradation, little Methyl formate is expected to survive a drainage system and wastewater treatment plant

(unless the concentration is suffiently high to affect the pH after initial hydrolsis). What little amount that remains in the effluent will undergo hydrolysis to methanol and formic acid, both of which are hydrolytically stable. Therefore, the effects of methyl formate on aquatic species are better estimated by using data from methanol and formic acid. Furthermore, as the limited experimental data available support the use of the "esters" ECOSAR QSAR model, it is concluded that the QSAR estimate for the toxicity of methyl formate to aquatic species is adequate for the purposes of the EPA HPV program.

*Appendix II: Methanol Developmental Toxicity Summaries**

* Summaries from Methanol HPV Document

Species:	rat
Sex:	female
Strain:	Sprague-Dawley
Route of admin.:	inhalation
Exposure period:	7 hours/day
Frequency of treatment:	daily
Duration of test:	day 1-19 of gestation
Doses:	0, 5000, 10000, 20000 ppm
Control Group:	yes, concurrent vehicle
NOAEL Maternal Toxicity:	>= 10000 ppm
NOAEL Teratogenicity:	= 10000 ppm
LOAEL Maternal Toxicity:	<= 20000 ppm
LOAEL Teratogenicity :	<= 20000 ppm
Method:	other
Year:	1985
GLP:	no

Test substance:

Reagent Grade Methanol -Matheson, Colemen & Bell Manuf. Chemists

Method:

Pregnant females, 15 per group, except 13/ group at 5,000 ppm were exposed for 7 hrs/ day, on days 1-19 of gestation.Parameters evaluated included body weight, food consumption, clinical signs, and survival. Blood methanol level, corpora lutea, dead, resorbed, malformed fetuses [half visceral, half skeletal] observations were also made.

Remark:

Different incidences of visceral malformations were reported in the text than were reported in the tables. The occurrence of maternal toxicity in the significantly affected group compromises an interpretation of the teratogenic effects as being solely the result of in utero methanol exposure.

Result:

The highest concentration of methanol produced slight maternal toxicity (unsteady gait) and a high incidence of congenital malformations, predominantly extra or rudimentary cervical ribs and urinary or cardiovascular defects. Blood methanol increased with dose (1.00, 2.24 and 8.65 mg/ml}. The fetal observations that were significantly different from control ($p<0.05$) at 20,000 ppm are: Number of litters(fetus) with visceral and skeletal malformation, the percentage of litters with abnormal fetuses and the percentage of normal fetus. A non statistical increase in malformation was also reported at 10,000 ppm. No significantly differences from control ($p<0.05$) was noted at any of the lower doses.

Conclusion:

The highest level of methanol produced slight maternal toxicity and a significant increase in congenital malformations. A non-statistical increase in malformation was also reported at 10,000 ppm

Reliability: (2) valid with restrictions

Reference

Nelson, B.K., W.S. Brightwell, D.R. MacKenzie, A. Khan, J.R. Burg, W.W. Weigel, and P.T. Goad. (1985). Teratological Assessment of methanol and ethanol at high inhalation levels in rats. Fundamental and Applied Toxicol 5: 727-736.

Species:	rat
Sex:	female
Strain:	Sprague-Dawley
Route of admin.:	inhalation
Exposure period:	20 hours/day
Frequency of treatment:	daily
Duration of test:	day 7-17 of gestation
Doses:	0, 200, 1000, 5000 ppm
Control Group:	yes
NOAEL Maternal Toxicity:	> 1000 ppm
NOAEL Teratogenicity:	> 1000 ppm
LOAEL Maternal Toxicity :	<= 5000 ppm
LOAEL Teratogenicity :	<= 5000 ppm
NOAEL Embryotoxicity :	>= 1000 ppm

Method:	other
Year:	1986
GLP:	no data
Test substance:	Methanol, Junsei Chemical Co.

Method:

Thirty-six pregnant rats per group were used in this study.Twenty were sacrificed/group at day 20 and 10/group were allowed normal delivery. Parameters evaluated included body weight, food consumption, clinical signs, organ weight, histopathology and survival. The number of fertilized corpus luteum, implantation, living and death fetuses, were also determined. Visceral or skeleton evaluations were conducted on each litter. Offspring from the normal delivery were subjected to general observation at birth, organ check, organ texture, genital function and movement. The F1 were bred and all sacrificed at day 20, litter evaluated as above.

Remark:

Similar to standard teratogenicity study in rats except the length of the daily exposure was much longer than normal (20 vs 6 hrs little time for clearance as exposure was essentially continuous) and some pregnant rats were allowed to deliver normally.

Result:

Dams exposed at 5,000 ppm had decreased rate of body weight gain, decreased food/water consumption, and one death (plus one sacrificed). Increased embryo mortality, decreased fetal weight, increase in septal defects, obstructed vertebro-costal foramen, cervical ribs, excess sublingual foramen nervosa, delayed fetal growth and reduced ossification were noted in the high dose fetuses. In the normal delivery groups decreased food/water consumption, and delayed pregnancy (0.7 days) were reported in the high dose. Pups from the high dose group had increased early mortality, lower birth weights, lower postnatal body weight and a slight decreased water consumption (no effect on food consumption). The high dose male offspring sacrificed at 8 weeks had decreased brain, thyroid, thymus, testes weight and increased in hypohysis weight. The high dose females had reduced brain and thymus weights. No histopathological effects were noted in these organs, except hemilateral thyroid defects

Conclusion:

Inhalation exposure demonstrated treatment-related effects in rats and their fetuses exposed at 5,000 ppm. Fetal toxicity, visceral/skeleton effects were seen in the fetuses exposed at 5,000 ppm , but this dose was maternally toxic. Methanol is not considered teratogenic in this study. 1,000 ppm was a NOAEL for both the dam and the fetus.

Reliability: (2) valid with restrictions

Reference

New Energy Development Organization (NEDO). (1986). Toxicological research of methanol as a fuel for power station. Tokyo, Japan. September.

Species:	mouse
Sex:	female
Strain:	CD-1
Route of admin.:	inhalation
Exposure period:	7 hours/day
Frequency of treatment:	day 6-15 of gestation
Duration of test:	20 days
Doses:	0, 1000, 2000, 5000, 7500, 10000, 15000 ppm
Control Group:	yes
NOAEL Maternal Toxicity:	> 15000 ppm
NOAEL Teratogenicity:	= 1000 ppm
NOAEL Embryotoxicity :	= 1000 ppm
LOAEL Teratogenicity :	= 2000 ppm
LOAEL Embryotoxicity :	= 2000 ppm
Method:	other
Year:	1993
GLP:	no data
Test substance:	Methanol , High purity Optima grade Fisher Scientific

Method:

Blood methanol concentrations were determined on gestation days 6, 10, and 15. Fetus were examined for number of implantation sites, live/dead fetuses, and resorptions. Fetuses were examined externally and weighed as a litter. Half of each litter was examined for skeletal morphology/ Internal soft tissue anomalies.

Remark:

Study design is complex. Only 3 chambers were used and exposures were staggered. Number of animal used appeared to be adequate, but it was hard to tell exact number used, as data was listed as litters per treatment group. Some animals were removed for plasma methanol determinations.

Result:

One dam died in each of the 7,500, 10,000, and 15,000 ppm methanol exposure groups. The methanol exposed dams gained

less weight than did unexposed dams fed ad libitum. Significant increases in the incidence of exencephaly and cleft palate were observed at 5,000 ppm and above, increased embryo/fetal death at 7,500 ppm and above (including an increasing incidence of full- litter resorptions), and reduced fetal weight at 10,000 ppm and above. A dose-related increase in cervical ribs or ossification sites lateral to the seventh cervical vertebra was significant at 2,000 ppm and above. Methanol plasma levels increased with dose. No signs of maternal toxicity were noted.

Conclusion:

The NOAEL for the developmental toxicity in this study was 1,000 ppm.

Reliability: (2) valid with restrictions

Reference:

Rogers, J.M.; M.L., Mole; N., Chernoff; B.D.,Barbee; C.L., Turner; T.R., Logsdon; R.J.,Kavlock, (1993), The developmental toxicity of inhaled methanol in the CD-1 mouse, with quantitative dose-response modeling for estimation of benchmark doses. Teratology 47(3):175-88

Species:	mouse Sex: female
Strain:	CD-1
Route of admin.:	inhalation
Exposure period:	6 hours/day
Frequency of treatment:	7-9 and day 9-11, day 6-15
Duration of test:	20 days
Doses:	0, 5000, 10000, 15000 ppm
Control Group:	yes
NOAEL Maternal Toxicity:	>= 10000 ppm
NOAEL Teratogenicity:	= 5000 ppm
LOAEL Maternal Toxicity :	<= 15000 ppm
LOAEL Teratogenicity :	= 10000 ppm

NOAEL Embryotoxicity : = 5000 ppm

Method: other

Year: 1993

GLP: no data

Test substance: Methanol -HPLC grade J.T. Baker

Method:

The study used 17-27 pregnant mice per group. Parameters evaluated include body weight, clinical signs, and survival. The number of live/ dead fetuses, resorbed, and malformed fetuses[visceral] plus implant sites was also determined.

Remark:

A pilot study was also reported in this paper that was used to set conditions for the main study. A good special study that examined certain time periods during gestation and the effect of methanol on neural tube defects.

Result:

Neurological effects (ataxia, depressed motor activity circling, tilted heads) were noted in the 15,000 ppm dams only on days 1,2 and 3 of exposure (20, 10, 5%). Maternal body weights were decreased at day 17 at 15,000 ppm (high resorptions). Embryotoxicity was noted in fetuses (increased resorptions, reduced fetal weights, and/or fetal malformations) at 10,000 and 15,000 ppm level, while no observable adverse effects were seen in the 5,000 ppm group. Neural and ocular defects, cleft palate, hydronephrosis, deformed tails, and limb (paw and digit) anomalies were reported. Neural tube defects and ocular lesions occurred after methanol inhalation between GD 7-9, while limb anomalies were induced only during GD 9-11, cleft palate and hydronephrosis were observed after exposure during either period.

Conclusion:

The highest level of methanol (15,000 ppm) produced slight maternal toxicity and embryotoxicity. Teratogenicity and embryotoxicity was also reported at 10,000 ppm, but not 5,000 ppm.

Reliability: (2) valid with restrictions

Reference:

Bolon, B., D.C. Dorman, D. Janszen, K.T. Morgan, and F. Welsch. (1993). Phase-specific developmental toxicity in mice following maternal methanol inhalation. Fund. Appl. Toxicol.21:508-516.

Species: monkey Sex: female

Strain: Macaca Fascicularis

Route of admin.:	inhalation
Exposure period:	2.5 hours/day
Frequency of treatment:	daily
Duration of test:	120 days
Doses:	0, 200, 600, 1800 ppm
Control Group:	yes, concurrent vehicle
NOAEL Maternal Toxicity:	> 1800 ppm
NOAEL Teratogenicity:	> 1800 ppm
Method:	other
Year:	1999
GLP:	no data
Test substance:	Methanol , High purity HPLC grade Fisher Scientific

Method:

Eleven or 12 female monkey per group were used in this study. Pregnancy observations and delivery exam were conducted. In addition body weight, clinical signs, and survival were also evaluated.

Remark:

An excellent well conducted study in a species that is more closely associated with humans as far as methanol toxicity is concerned. Study was sponsored by EPA, auto companies and API. Study was reviewed by an outside expert panel, whoagreed with the study author's conclusions.

Result:

The weights of all females were quite stable during the study. Mean weight gain during pregnancy varied from 1.3 kg to 1.8 kg across all exposure groups. Clinical observations did not indicate the presence of overt toxicity in the adult females, and none exhibited a pattern of responses indicative of fine-motor incoordination due to methanol exposure. Methanol exposures were associated with a reduction in the length of pregnancy, thus shortening the gestation period of the offspring, but did not affect the size of the offspring at birth, the average birth weight, crown-rump length, and head size of infants in the methanol-exposure groups. Neurobehavioral testing suggested possible effects in two tests, but the effects was not concentration dependant or a significant overall effect across the methanol groups. Another observation, not judged a treatment related effect, was a serve wasting syndrome in two female offspring in the high dose methanol group. These two observations were not considered to be treatment related.

Conclusion:

Chronic methanol exposures for up to 1 year did not cause overt maternal toxicity in m. fascicularis females. Methanol exposures were associated with a

reduction in the length of pregnancy (not dose dependent). No decrease in offspring birth size was observed. No obvious birth defects were noted.

Reliability: (1) valid without restriction

Reference:

Burbacher, T., D.,Shen, K.,Grant, L.,Sheppard, D.,Dumian, S.,Ellis andc N.,Liberato, (1999) Reproductive and offspring developmental effects following maternal inhalation exposure to methanol in non-human primates, Part 11: Developmental effects in infants exposed prenatally to methanol. Health Effects Institute Report 89 October 1999

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